

EXHIBIT F

DAY 0. Test before the first challenge.

RAL cell count 250µl

$$OX40^{-/-} = 41 \times 10^4 = 4.1 \times 10^5 \text{ cells/ml.}$$

$$OX40^{-/-} = 74 \times 10^4 = 7.4 \times 10^5 \text{ cells/ml.}$$

$$OX40^{+/+} = 75 \times 10^4 = 7.5 \times 10^5 \text{ cells/ml.}$$

$$OX40^{+/+} = 54 \times 10^4 = 5.4 \times 10^5 \text{ cells/ml.}$$

collagenase.

$$\frac{5 \text{ mg}}{50} \times 3000 = \underline{300 \mu\text{l.}}$$

$$\frac{0.1}{10} \times 3000 = \underline{30 \mu\text{l.}}$$

DMSO 40 min 37°C

thymic count

FTC8

$$2 \text{ mice/group } OX40^{-/-} (2 \text{ ml}) \text{ LN} = 67 \times 2 \times 10^4 = 1.34 \times 10^6 \text{ cells/ml.} \quad (2 \text{ ml})$$

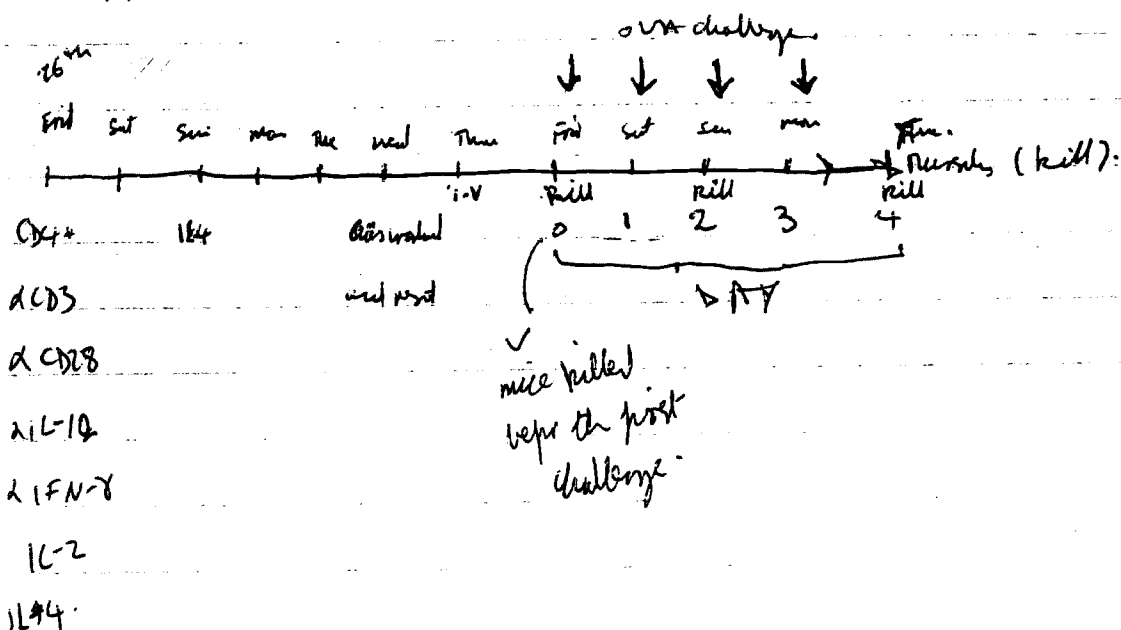
$$(10 \text{ ml}) \text{ LUNG} = 139 \times 2 \times 10^4 = 2.78 \times 10^6 \text{ cells/ml.} \quad (4 \text{ ml})$$

$$(10 \text{ ml}) \text{ Spleen} = 1036 \times 2 \times 10^4 = 2.072 \times 10^7 \text{ cells/ml.} \quad (1 \text{ ml})$$

$$2 \text{ mice/group } OX40^{+/+} (2 \text{ ml}) \text{ LN} = 45 \times 2 \times 10^4 = 9.0 \times 10^5 \text{ cells/ml.} \quad (2 \text{ ml})$$

$$(10 \text{ ml}) \text{ LUNG} = 102 \times 2 \times 10^4 = 2.04 \times 10^6 \text{ cells/ml.} \quad (4 \text{ ml})$$

$$(10 \text{ ml}) \text{ Spleen} = 625 \times 2 \times 10^4 = 2.080 \times 10^7 \text{ cells/ml.} \quad (1 \text{ ml})$$



DAY (2)

BAC cell count \Rightarrow 250 μ l.

OX40^{-/-} control = $55 \times 10^4 = 5.5 \times 10^5$

" " #1 = ~~15~~ $15 \times 10^4 = 1.5 \times 10^5$

#2 = $45 \times 10^4 = 4.5 \times 10^5$

#3 = $8 \times 10^4 = 0.8 \times 10^5$

#4 = $17 \times 10^4 = 1.7 \times 10^5$

OX40^{+/+} control = $20 \times 10^4 = 2 \times 10^5$

#1 = $114 \times 10^4 = 1.14 \times 10^6$

#2 = $103 \times 10^4 = 1.03 \times 10^6$

#3 = $123 \times 10^4 = 1.23 \times 10^6$

#4 = $115 \times 10^4 = 1.15 \times 10^6$

colony = $\frac{5}{50} \times 5000 = 500$

$\frac{0.1}{10} \times 500 = 50$

live cell count =

(FACS)

1 max. { OX40^{-/-} control = (1ml) LN = $210 \times 2 \times 10^4$
 (15ml) LUNG = $37 \times 5 \times 10^4$
 (15ml) spleen = $133 \times 5 \times 10^4$
 4 max. (2ml) #1-4 LN = $83 \times 2 \times 10^4$
 3 max. (15ml) #2-4 LUNG = $142 \times 5 \times 10^4$
 2 max. (15ml) #3-4 spleen = $273 \times 5 \times 10^4$

(1ml) 420×10^6 cells/ml
 (4ml) 1.85×10^6 cells/ml
 (1ml) 6.65×10^6 cells/ml
 (2ml) 740×10^6
 (0.5ml) 710×10^6
 (0.5ml) 1.365×10^7

1 max. { OX40^{+/+} control (1ml) LN = $83 \times 2 \times 10^4 =$
 (15ml) LUNG = $36 \times 5 \times 10^4 =$
 (15ml) spleen = $141 \times 5 \times 10^4 =$
 (2ml) #1-4 LN = $258 \times 5 \times 10^4 =$
 (15ml) #2-4 LUNG = $156 \times 5 \times 10^4$
 (15ml) #3-4 spleen = $342 \times 5 \times 10^4$ F2

(1ml) 166×10^6
 (4ml) 1.80×10^6
 (1ml) 705×10^6
 (1ml) 1.425×10^7
 (1ml) 280×10^6
 (0.5ml) 1.70×10^7

Eosinophil cell count:-

<u>DAY 0</u>	$OX40^{-/-}$ #1	$OX40^{-/-}$ #2	$OX40^{+/+}$ #1	$OX40^{+/+}$ #2
Total:-	200	200	199	199
Eosinophil:-	0	0	1	1
%	= 0	= 0	= 0	= 0

<u>DAY 2</u>	$OX40^{-/-}$ Control	$OX40^{+/+}$ Control
Total:-	200	200
Eosinophil:-	0	0
%	= 0	= 0

$OX40^{-/-}$	#1	#2	#3	#4	$OX40^{+/+}$	#1	#2	#3	#4
Total	377	very few cells	209	265		463	510	605	468
count	27	-	28	66		263	202	261	303
%	= 7.16%	-	13.40%	24.91%		56.80%	39.61%	43.14%	64.75%

BAL cell count 280pl.

DAY 4

$$OX40^{-/-} \text{ control} = 64 \times 10^4 = 6.4 \times 10^5$$

$$\#1 = 194 \times 2 \times 10^4 = 3.88 \times 10^6$$

$$550 \times 250 = 5 \times 2$$

$$\#2 = 497 \times 2 = 9.94 \times 10^6$$

$$\#3 = 260 \times 2 = 5.20 \times 10^6$$

$$\#4 = 240 \times 2 = 4.80 \times 10^6$$

$$OX40^{+/+} \text{ control} = 65 \times 10^4 = 6.5 \times 10^5$$

$$\#1 = 286 \times 2 \times 10^4 = 5.72 \times 10^6$$

$$\#2 = 616 \times 2 \times 10^4 = 1.23 \times 10^7$$

$$\#3 = 576 \times 2 \times 10^4 = 1.15 \times 10^7$$

$$\#4 = 589 \times 2 \times 10^4 = 1.18 \times 10^7$$

liver count.

$$\text{Collagenase} = \frac{5}{200} \times 6000 = 300 \mu\text{l}$$

$$\frac{0.1}{10} \times 6000 = 60 \mu\text{l}$$

$$(1 \text{ ml}) \quad 1 \text{ min} \quad OX40^{-/-} = \text{control LN} = 68 \times 2 \times 10^4$$

(1 ml).

$$(5 \text{ ml}) \quad 1 \text{ min} \quad \text{LUNG} = 47 \times 2 \times 10^4$$

(4.5 ml).

$$(15 \text{ ml}) \quad 1 \text{ min} \quad \text{spleen} = 246 \times 2 \times 10^4$$

(1 ml).

$$(2 \text{ ml}) \quad 4 \text{ min} \quad OVA \text{ challenge. } \#1 - \#4 = \text{LN} = 700 \times 2 \times 10^4 =$$

(0.5 ml).

$$(15 \text{ ml}) \quad 3 \text{ min} \quad \#2,3,4 = \text{LUNG} = 227 \times 5 \times 10^4 =$$

(1 ml).

$$(15 \text{ ml}) \quad 3 \text{ min} \quad \#2,3,4 = \text{spleen} = 299 \times 5 \times 10^4 =$$

(0.5 ml).

$$(2 \text{ ml}) \quad 1 \text{ min} \quad OX40^{+/+} = \text{control} = 103 \times 2 \times 10^4$$

$$(15 \text{ ml}) \quad 1 \text{ min} \quad 43.4 = \text{LUNG} = 49 \times 2 \times 10^4$$

$$(15 \text{ ml}) \quad 1 \text{ min} \quad 127 = \text{spleen} = 129 \times 2 \times 10^4$$

$$(2 \text{ ml}) \quad 3 \text{ min} \quad OVA \text{ challenge. } 1,2,4 = \text{LN} = 620 \times 2 \times 10^4 =$$

(0.5 ml).

$$(15 \text{ ml}) \quad 3 \text{ min} \quad 2,3,4 = \text{LUNG} = 185 \times 5 \times 10^4 =$$

(1 ml).

$$(15 \text{ ml}) \quad 3 \text{ min} \quad 43.4 = \text{spleen} = 337 \times 5 \times 10^4 =$$

(0.5 ml).

FACS

Folden = BALF - FSC/SSC

DAY 4

BALF DAY 4 FSC/SSC

OX

Scanned

1. OK40⁻¹ C 0.01

2. # 1 0.02

3. # 2 0.03

4. # 3 0.04

5. # 4 0.05

6. OK40⁺ C 0.06

7. # 1 0.07

8. # 2 0.08

9. # 3 0.09

10. # 4 0.010

#5
#6

100

10%

10

DAY 4

AG 6756

Investigator: **Croft/Shahram**

Protocol: **AF3-MC1-032802** Rec'd:

Species: **Mouse**

Source: **Jax**

Strain: **C57BL/6J**

Sex: **Female**

DOB:

Date Weaned:

Dam:

Sire:

2002 2X10⁶ cells OT1: CX40 +/-

o/n challenge

o/n challenge

o/n challenge

- o/n challenge
killed

DAY 4

AG 6751

Investigator: **Croft/Shahram**

Protocol: **AF3-MC1-032802** Rec'd:

Species: **Mouse**

Source: **Jax**

Strain: **C57BL/6J**

Sex: **Female**

DOB:

Date Weaned:

Dam:

Sire:

2X10⁶ cells OT1: CX40 -/-

o/n challenge

o/n challenge

o/n challenge

- o/n challenge
killed

Solich 112: - Adoplin

Final Selling

100 1. CD4 APC

101 2. VK PE

102 3. VB Cys

103 4. CFSE

104 5. +/+ CFSE

105 6. -/- CFSE

106 7. control

107 8. CD4 APC

108 9. VK

109 10. VB

111 11. +/+ LN

112 12. +/+ LUNG

113 13. +/+ spleen

114 14. -/- LN

115 15. -/- LUNG

116 16. -/- spleen

117 17. +/+ LN

118 18. +/+ LUNG

119 19. +/+ spleen

120 20. +/+ LN

121 21. +/+ LUNG

122 22. +/+ spleen

123 23. -/- LN

124 24. -/- LUNG

125 25. -/- spleen

126 26. -/- LN

127 27. -/- LUNG

128 28. -/- spleen

Before surgery

DAY 0

Control

OVA

Control

OVA

DAY 2

2.29 (→ FSC → 2.05

481 (→ SSC → 462

489 (→ FL-1 = 600. 474 485

526 (→ FL-2 574 520 485 526

719 (→ FL-3 = 763 700 718

966 (→ FL-4 840 826 90

FL-1 0.5 FL-2

FL-2 26.5 FL-1

FL-2 0.6 FL-3

FL-3 24.2 FL-2 25.4

FL-3 23.6 FL-4

FL-4 0. FL-3

29. +/+ LN

30. +/+ LUNG

31. +/+ spleen

32. +/+ LN

33. +/+ LUNG

34. +/+ spleen

35. -/- LN

36. -/- LUNG

37. -/- spleen

38. -/- LN

39. -/- LUNG

40. -/- spleen

Control

OVA

Control

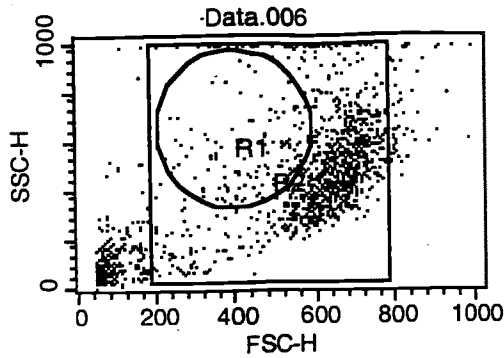
OVA

DAY 4

DAY 2 Total # of VαVβ positive CD4 cells.

				DAY 0	DAY 2.
DAY 2	+/+ LN	PBS		2.5650×10^4	7.40×10^4 6.5
	+/+ LN	OVA.			4.73×10^5
	-/- LN	PBS		2.6667×10^4	3.18×10^5
DAY 2	-/- LN	OVA.			2.80×10^5
DAY 2	+/+ LUNG	PBS		2.93760×10^5	1.19×10^6
	+/+ LUNG	OVA			2.99×10^6 7.5
	-/- LUNG	PBS		5.27×10^5	3.05 3.05×10^6
	-/- LUNG	OVA.			3.09×10^6
DAY 2	+/+ spleen	PBS		1.84×10^6	4.66×10^6
	+/+ spleen	OVA			1.40×10^7 3-1
	-/- spleen	PBS		2.76×10^6	5.137 5.137×10^6
	-/- spleen	OVA.			7.32×10^6
DAY 2					

BALF DAY 4



File: Data.006

Acquisition Date:

Gated Events: 4449

X Parameter: FSC-H (Linear)

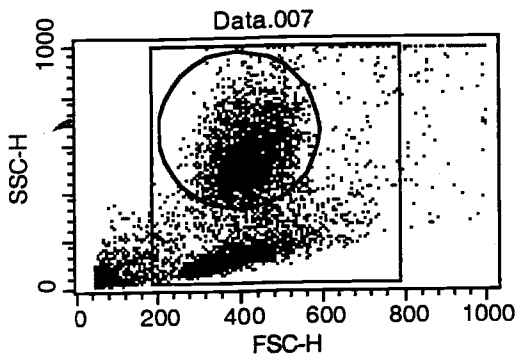
Sample ID: 6 (OX40⁺)

Gate: No Gate

Total Events: 4449

Y Parameter: SSC-H (Linear)

Region	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
R1	371	8.34	8.34	443.40	538.14
R2	3433	77.16	77.16	580.35	453.94



File: Data.007

Acquisition Date:

Gated Events: 20000

X Parameter: FSC-H (Linear)

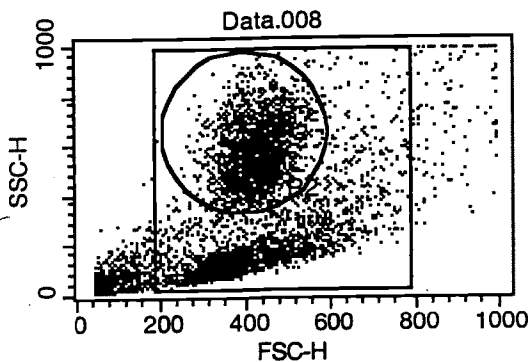
Sample ID: 7 (OX40^{+/+})

Gate: No Gate

Total Events: 20000

Y Parameter: SSC-H (Linear)

Region	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
R1	9582	47.91	47.91	416.11	547.33
R2	17592	87.96	87.96	402.27	303.17



File: Data.008

Acquisition Date:

Gated Events: 20000

X Parameter: FSC-H (Linear)

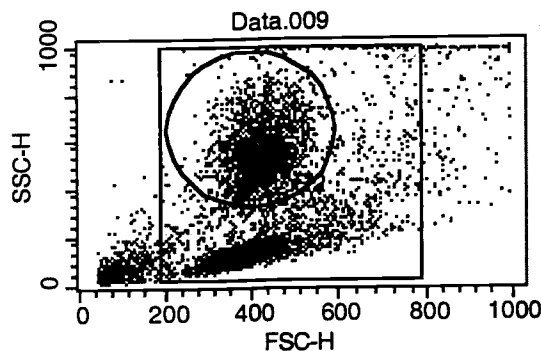
Sample ID: 8 (OX40^{+/+})

Gate: No Gate

Total Events: 20000

Y Parameter: SSC-H (Linear)

Region	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
R1	7624	38.12	38.12	420.18	558.87
R2	17364	86.82	86.82	401.27	265.46



File: Data.009

Acquisition Date:

Gated Events: 20000

X Parameter: FSC-H (Linear)

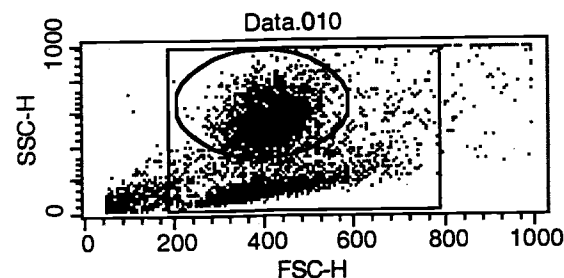
Sample ID: 9 (OX40^{+/+})

Gate: No Gate

Total Events: 20000

Y Parameter: SSC-H (Linear)

Region	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
R1	7846	39.23	39.23	417.90	547.84
R2	17594	87.97	87.97	403.40	267.84



File: Data.010

Acquisition Date:

Gated Events: 20000

X Parameter: FSC-H (Linear)

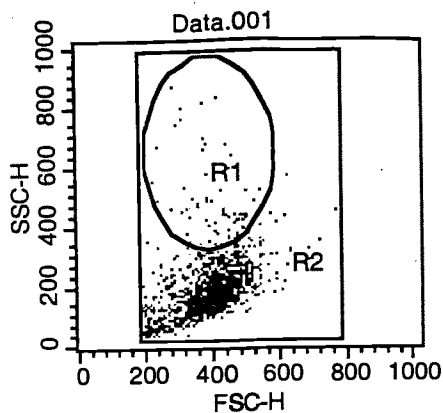
Sample ID: 10 (OX40^{+/+})

Gate: No Gate

Total Events: 20000

Y Parameter: SSC-H (Linear)

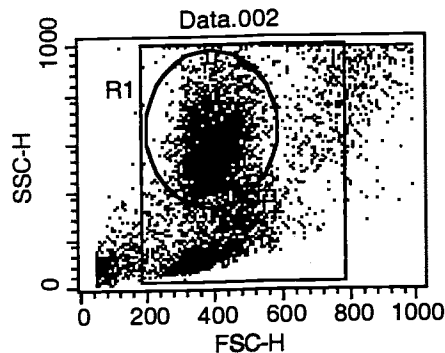
Region	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
R1	9063	45.32	45.32	410.59	545.45
R2	17510	87.55	87.55	400.01	298.35



File: Data.001 Sample ID: 1
 Acquisition Date: Gate: G2
 Gated Events: 1355 Total Events: 2540
 X Parameter: FSC-H (Linear) Y Parameter: SSC-H (Linear)

Region	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
R1	73	5.39	2.87	399.10	488.51
R2	1355	100.00	53.35	384.48	178.22

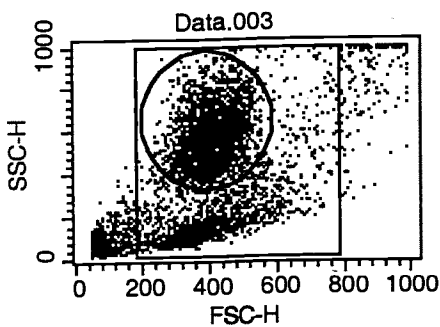
0.



File: Data.002 Sample ID: 2 (OX40-/-)
 Acquisition Date: Gate: No Gate
 Gated Events: 20000 Total Events: 20000
 X Parameter: FSC-H (Linear) Y Parameter: SSC-H (Linear)

Region	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
R1	9862	49.31	49.31	399.58	570.60
R2	17177	85.89	85.89	405.86	369.43

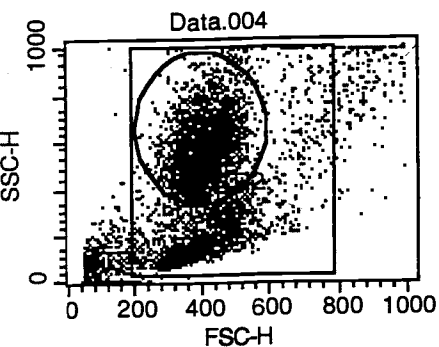
57.4%



File: Data.003 Sample ID: 3 (OX40-/-)
 Acquisition Date: Gate: No Gate
 Gated Events: 20000 Total Events: 20000
 X Parameter: FSC-H (Linear) Y Parameter: SSC-H (Linear)

Region	Events	% Gated	% Total	X Geo Mean	Y Geo Mean
R1	9881	49.41	49.41	409.32	565.83
R2	17406	87.03	87.03	397.05	325.51

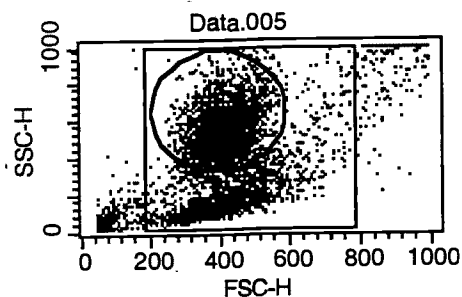
56.90



File: Data.004 Sample ID: 4 (OX40-/-)
 Patient ID: Acquisition Date:
 Gate: No Gate Gated Events: 20000
 Total Events: 20000

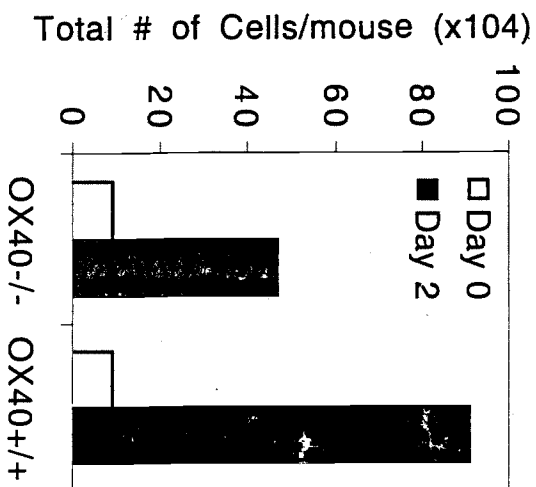
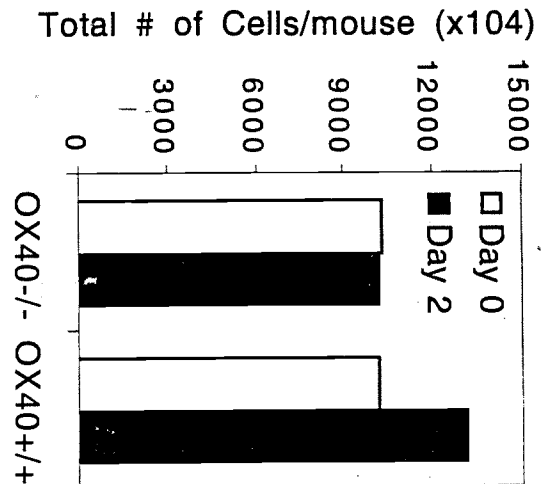
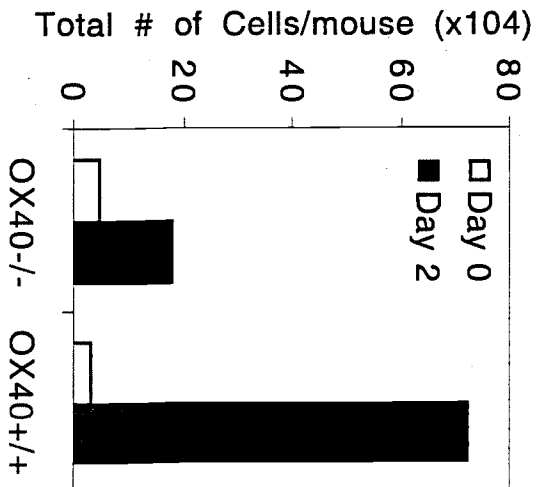
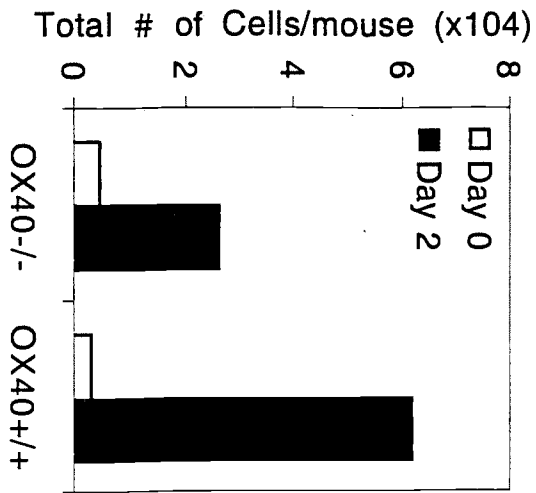
Region	Events	% Gated	% Total	X Geo Mean
R1	10083	50.42	50.42	402.54
R2	17120	85.60	85.60	401.14

50.90



File: Data.005 Sample ID: 5 (OX40-/-)
 Acquisition Date: Gate: No Gate
 Gated Events: 20000 Total Events: 20000
 X Parameter: FSC-H (Linear) Y Parameter: SSC-H (Linear)

Region	% Gated	% Total	X Geo Mean	Y Geo Mean
R1	49.53	49.53	406.11	536.70
R2	90.44	90.44	403.24	314.07

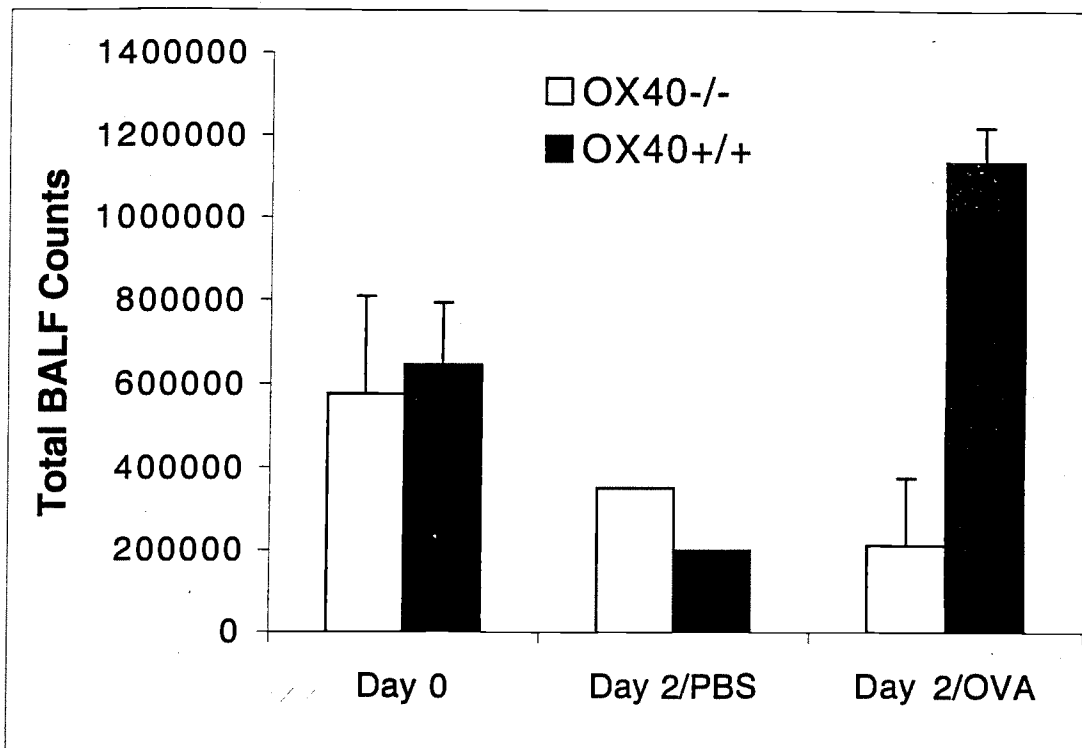


Total

DAY 0

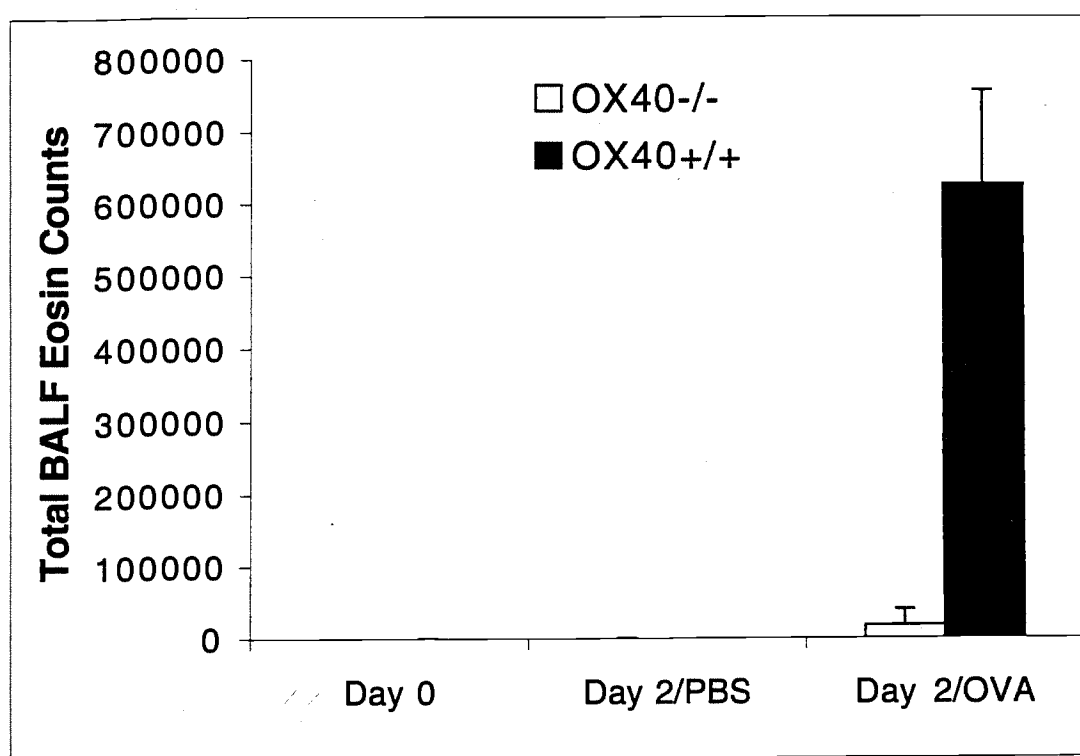
Day 0		Day 2			
OX40-/-	OX40+/+	OX40-/-	OX40+/+	OX40-/-	OX40+/+
410000	750000	350000	200000	150000	1140000
740000	540000			450000	1030000
				80000	1230000
				170000	1150000
575000	645000	350000	200000	212500	1137500
233345.238	148492.424	0	0	162967.277	82209.083

	Day 0	Day 2/PBS	Day 2/OVA		
OX40-/-	575000	350000	212500	233345	0
OX40+/+	645000	200000	1137500	148492	0



OX40-/-			OX40+/+		
Eosinophils%			Eosinophils%		
#1	#3	#4	#1	#3	#4
150000	80000	170000	1140000	1230000	1150000
7.16	13.4	24.91	56.8	39.61	64.75
10740	10720	42347	647520	487203	744625

	Day 0	Day 2/PBS	Day 2/OVA	
OX40-/-	0	0	18053	0
OX40+/+	0	0	626449	0

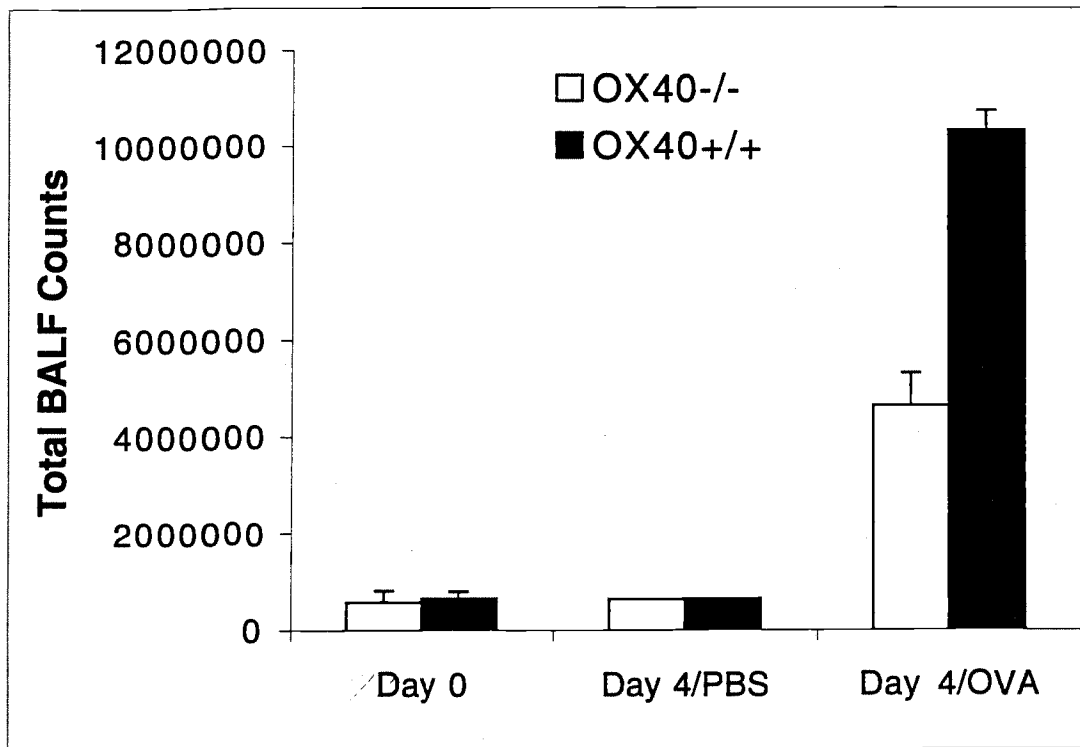


Tot

DAY(9)

Day0		Day 2			
OX40-/-	OX40+/+	OX40-/-	OX40+/+	OX40-/-	OX40+/+ --
410000	750000	640000	650000	3880000	
740000	540000				12300000
				5200000	11500000
				4800000	11800000
575000	645000	640000	650000	4626666.67	11866666.7
233345.238	148492.424	0	0	676855.474	404145.188

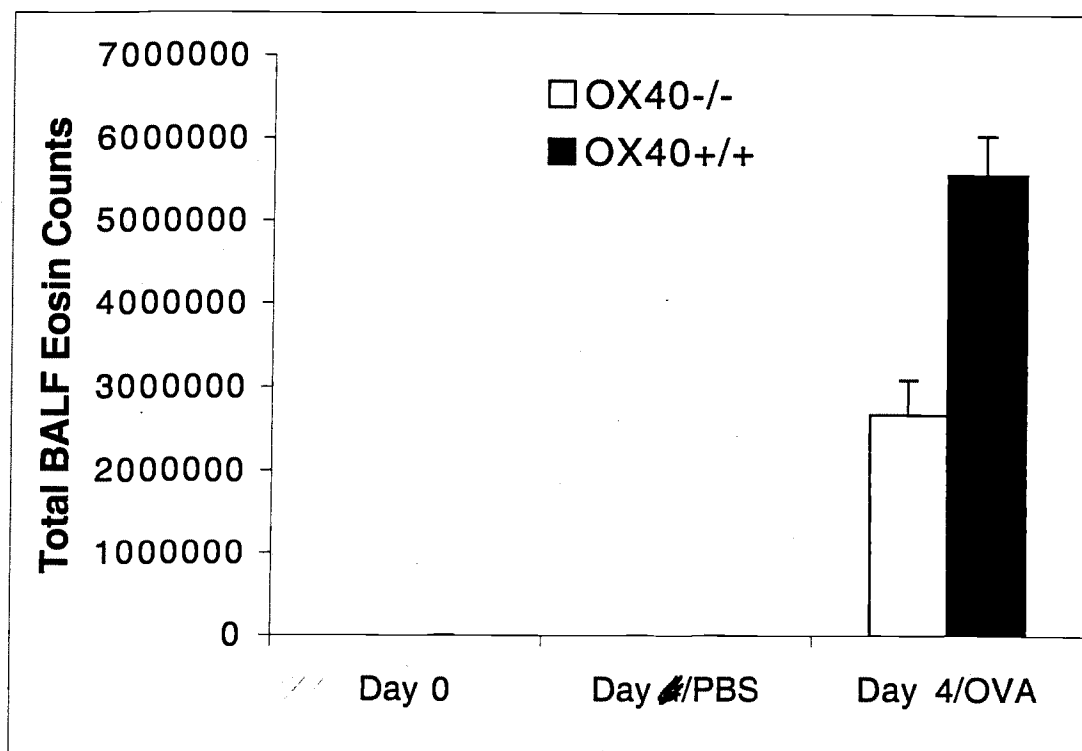
	Day 0	Day 4/PBS	Day 4/OVA		
OX40-/-	575000	640000	4626666	233345	0
OX40+/+	645000	650000	10330000	148492	0



Eosin DAY 4

OX40-/-			OX40+/+		
Eosinophils%			Eosinophils%		
#1	#3	#4	#2	#3	#4
3880000	5200000	4800000	12300000	11500000	11800000
57.4	58.9	56.8	44	45	51.76
2227120	3062800	2726400	5412000	5175000	6107680

	Day 0	Day 4/PBS	Day 4/OVA	
OX40-/-	0	0	2672107	0
OX40+/+	0	0	5564893	0



OX40 ^{-/-}			OX40 ^{+/+}		
#1	Eosinophils% #3	#4	#1	Eosinophils% #3	#4
150000	80000	170000	1140000	1230000	1150000
7.16	13.4	24.91	56.8	39.61	64.75
10740	10720	42347	647520	487203	744625

Day 2	OX40 ^{-/-}	OX40 ^{+/+}	Day 4	OX40 ^{-/-}	OX40 ^{+/+}
	7.16	56.8		57.4	54.45
	13.4	39.61		56.8	44
	24.91	64.75		58.9	51.76
	15.1566667	53.72		57.7	50.07
	9.0044452	12.8498911		1.08166538	5.42611279
	OX40 ^{-/-}	OX40 ^{+/+}	9	12.85	
Day 2	15.16	53.72	1	5.42	
Day 4	57.7	50.07			

